

Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figure Legends

Figure S1. Collagen-induced DDR1 ectodomain shedding is insensitive to TIMPs. (A) HEK293 cells transiently transfected with N-terminally FLAG-tagged DDR1 (DDR1-NF) were stimulated with collagen I in the presence or absence of 10 μ M Marimastat (Mst) and TIMP-1 or -2 at the concentration of 500 nM. Conditioned media and cell lysates were subjected to Western Blotting analysis using anti-DDR1 ectodomain (DDR1, Med), anti-DDR1 C-terminal domain (DDR1, Cell) or anti-actin antibodies. TIMP-1 and -2 in conditioned media were detected with sheep anti-TIMP-1 or -2 antiserum. CTF, C-terminal fragment. (B) A431 cells were stimulated with collagen I in the presence or absence of TIMP-1 or -2 at the concentration of 500 nM. Conditioned media and cell lysates were analyzed by Western Blotting as in A. (C) HEK293 cells stably expressing C-terminally FLAG-tagged full-length TIMP-3 or control cells (Mock) were used in the experiments. The cells were transiently transfected with empty vector (Mock) or DDR1-NF and stimulated with 100 μ g/ml collagen I in serum-free DMEM for 24 h in the presence or absence of 10 μ M Mst. Conditioned media and cell lysates were analyzed by Western Blotting with anti-DDR1 ectodomain (DDR1, Med), anti-DDR1-C (DDR1, Cell), anti-FLAG (TIMP-3, Med and Cell) or anti-actin antibodies. (D) HEK293 cells were transiently transfected with DDR1-NF and/or full-length TIMP-3, and cells were treated with collagen as in D. Conditioned media and cell lysates were subjected to Western Blotting analysis using anti-DDR1 ectodomain (DDR1, Med), anti-DDR1-C (DDR1, Cell), anti-TIMP-3 clone136-13H4 (TIMP-3, Med and Cell) or anti-actin antibodies.

Figure S2. Collagen-induced shedding of DDR1 in MCF-7 was inhibited by ADAM10 knockdown. MCF-7 cells were transfected for 48 h with siRNA targeting ADAM10 (si-A10), ADAM17 (si-A17) or non-targeting siRNA (si-NT). Cells were stimulated with collagen I. Conditioned media and cell lysates were analyzed by Western Blotting with anti-DDR1 ectodomain, anti-DDR1-C, anti-ADAM10 (A10), anti-ADAM17 (A17) or anti-actin antibodies. Relative intensities of shed DDR1 normalized to actin were standardized to si-NT samples treated with collagen. Data are shown in the bottom of the top panel. Asterisk indicates non-specific bands. Pro, proform of ADAM17; Active, active form of ADAM10 or ADAM17.

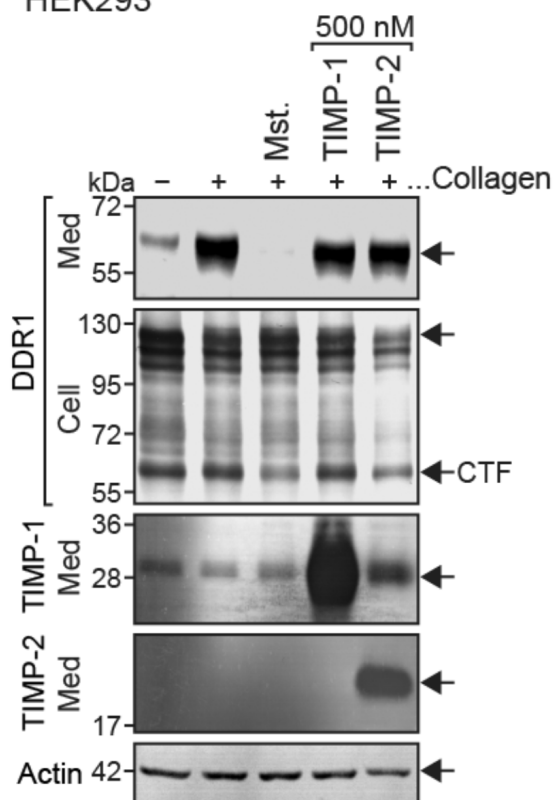
Figure S3. Ionomycin-induced DDR1 shedding in A431 was sensitive to ADAM10 knockdown. A431 cells were transfected with siRNA for ADAM10 (si-A10), ADAM17 (si-A17) or non-targeting siRNA (si-NT). Cells were then treated with 1 μ M IM or DMSO vehicle control for 1 h. Conditioned media were subjected to Western Blotting using anti-DDR1 ectodomain (Med). Cell lysates were analyzed by Western Blotting using anti-DDR1 C-terminus (DDR1, Cell), anti-ADAM10 (A10), anti-ADAM17 (A17) and anti-actin antibodies. The relative intensity of shed DDR1 are shown at the bottom of the upper panel. Pro, pro-form of ADAMs; Active, active form of ADAMs.

Figure S4. DDR1 mutants express on the cell surface. (A and B) HEK293 cells were transfected with expression plasmids for DDR1 mutants as indicated. Cells were subjected to surface biotinylation and lysed in RIPA buffer, followed by streptavidin beads affinity precipitation. The cell surface proteins bound to the beads were analyzed by Western Blotting with anti-DDR1 ectodomain, anti-HA or anti-actin antibodies. Actin was blotted as a negative control. IP, immunoprecipitation. (C and D) Immunostaining for surface DDR1 mutants expressed in HEK293 cells. Cells were fixed and stained for cell surface DDR1 mutants (green) with anti-HA (C) or anti-FLAG (D) antibodies. Nuclei (blue) and actin (white) were also stained. Protein expression of DDR1 mutants was confirmed by Western Blotting. Bar: 50 μ m.

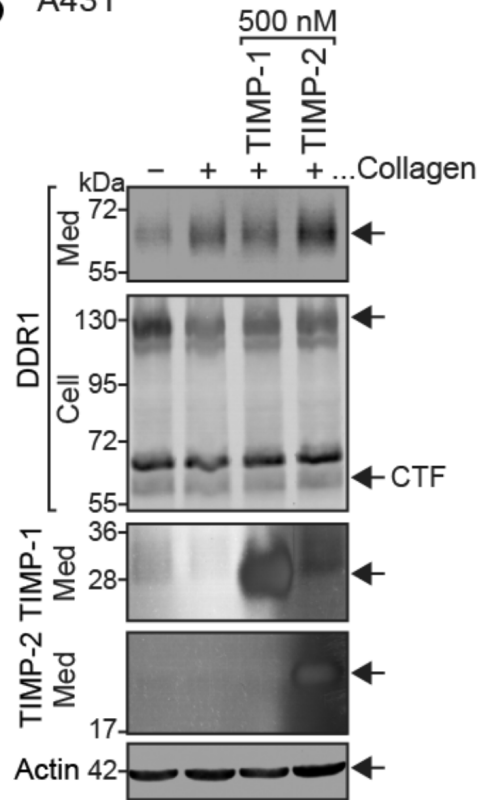
Figure S5. Effect of deletion of juxtamembrane region in DDR1 on its ectodomain shedding and phosphorylation triggered by collagen stimulation. (A) Schematic representation of mutant DDR1 constructs used in the experiments. Arrows indicate the cleavage sites identified. Numbers of amino acids are untagged wild type DDR1 numbering. S, signal

peptide; DD, discoidin-homology domain; DLD, discoidin-like domain, JM, juxtamembrane region; TM, transmembrane domain; TKD, tyrosine kinase domain; HA, HA-tag (YPYDVPDYA); FLAG, FLAG-tag (DYKDDDDK); KD, kinase dead; Δ C, cytoplasmic domain-deleted. (B and C) HEK293 cells were transfected with expression plasmids for DDR1 deletion mutants as indicated. Cells were incubated for 24 h (B) or 1 h (C) with 100 μ g/ml collagen. Conditioned media and cell lysates were subjected to Western Blotting using anti-DDR1 ectodomain, anti-actin and anti-phosphotyrosine 4G10 (PY) antibodies. Shedding of mutants was inhibited by addition of 10 μ M Marimastat (B, Mst).

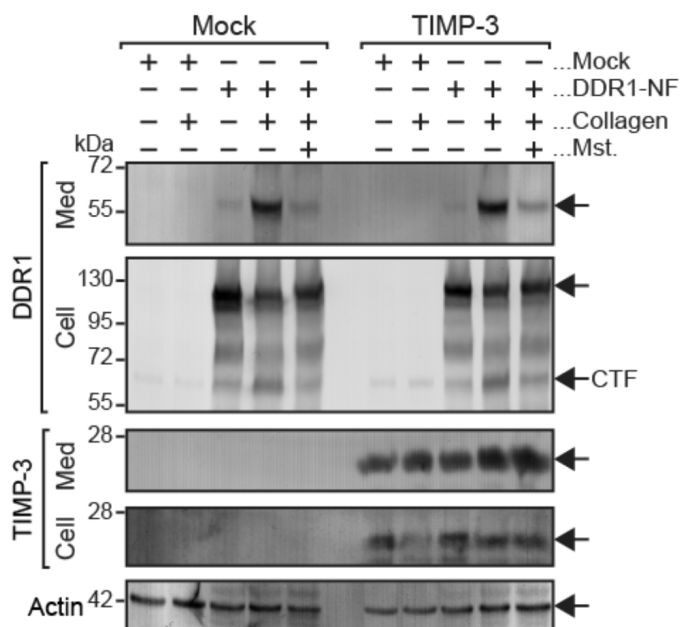
A HEK293



B A431



C HEK293 stable cell lines



D HEK293

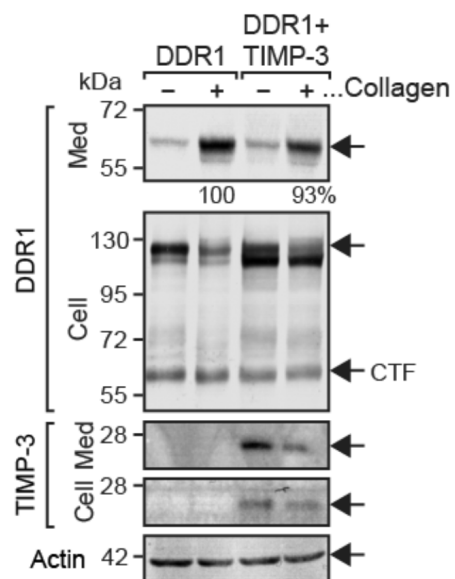


Figure S1

MCF-7

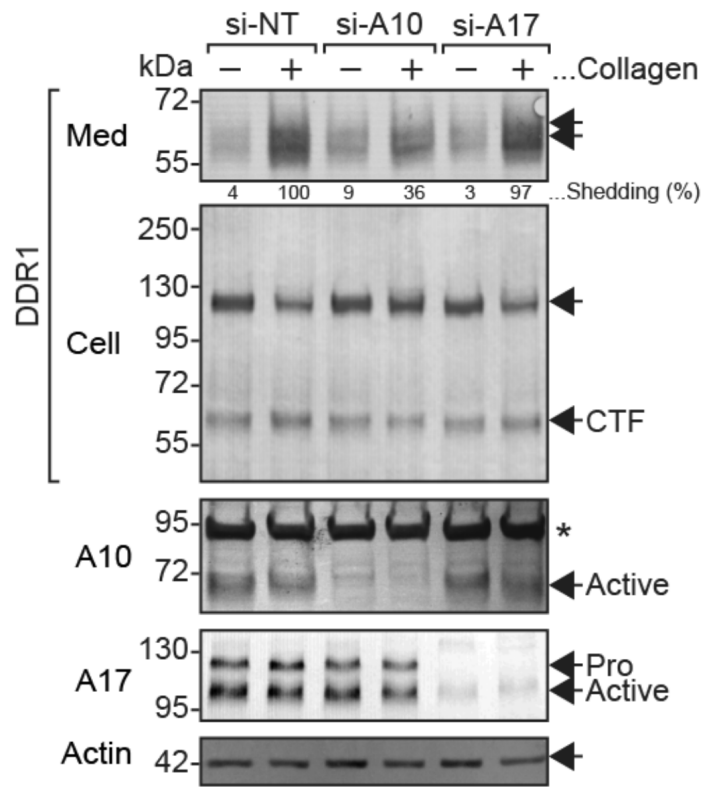


Figure S2

A431

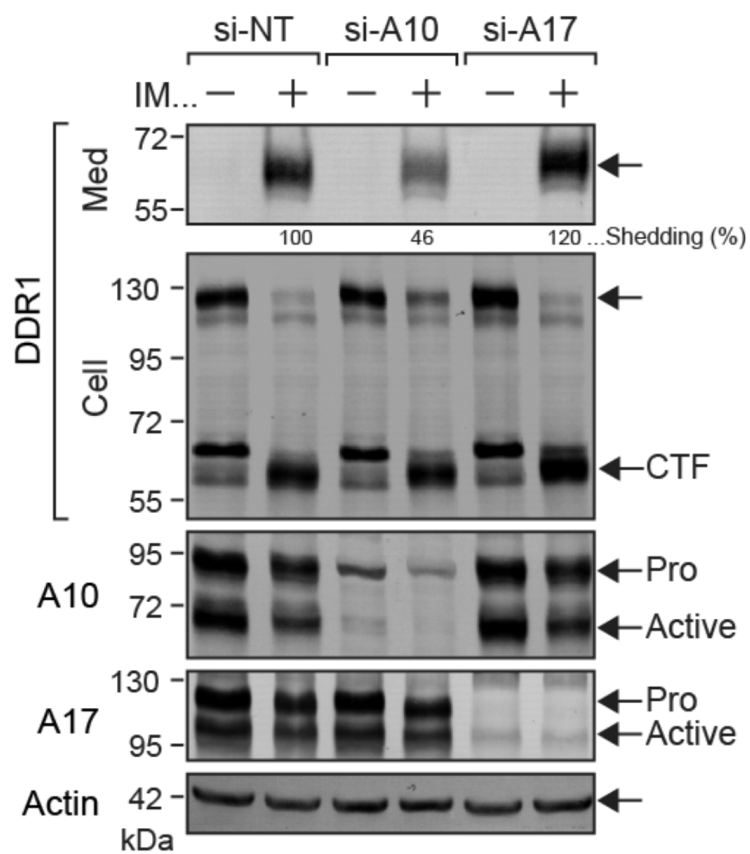


Figure S3

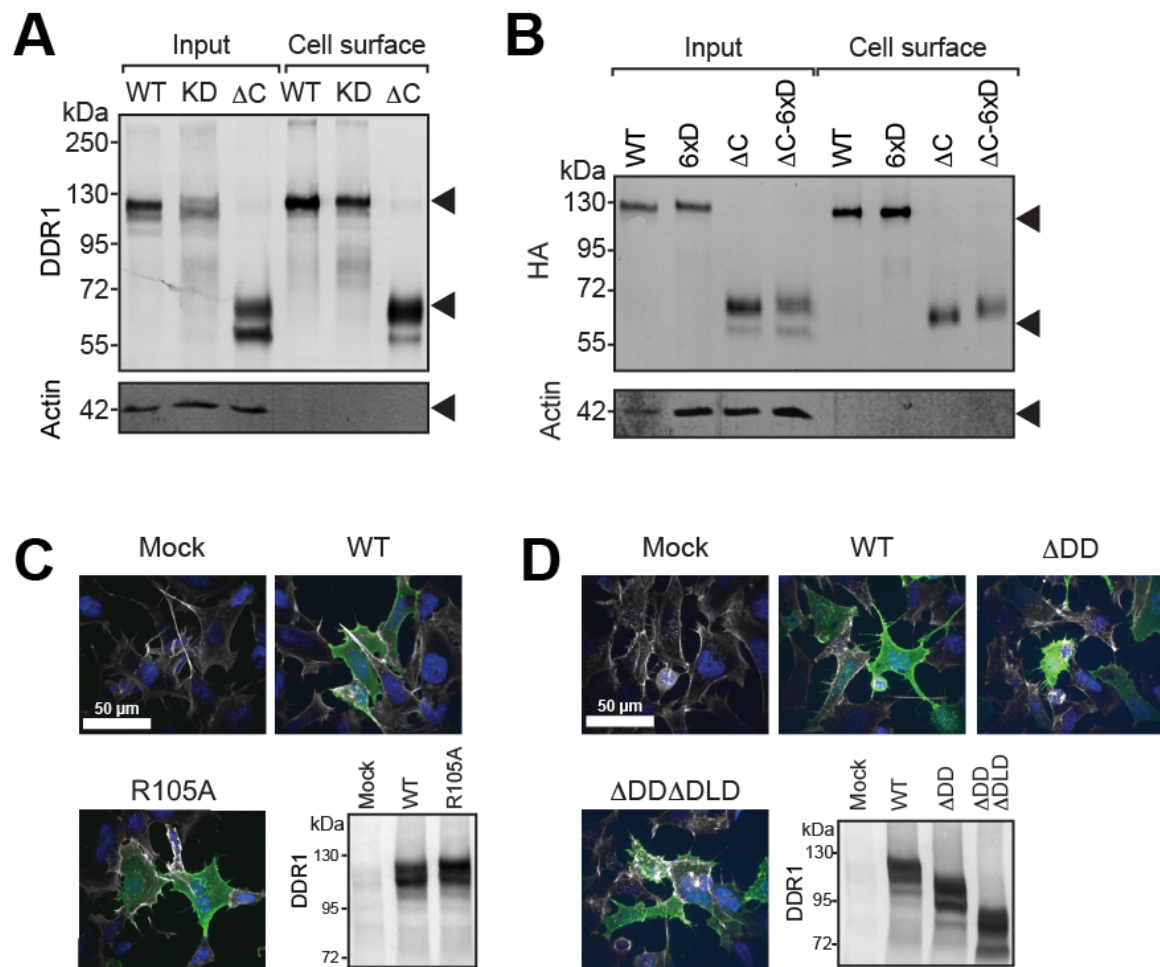


Figure S4

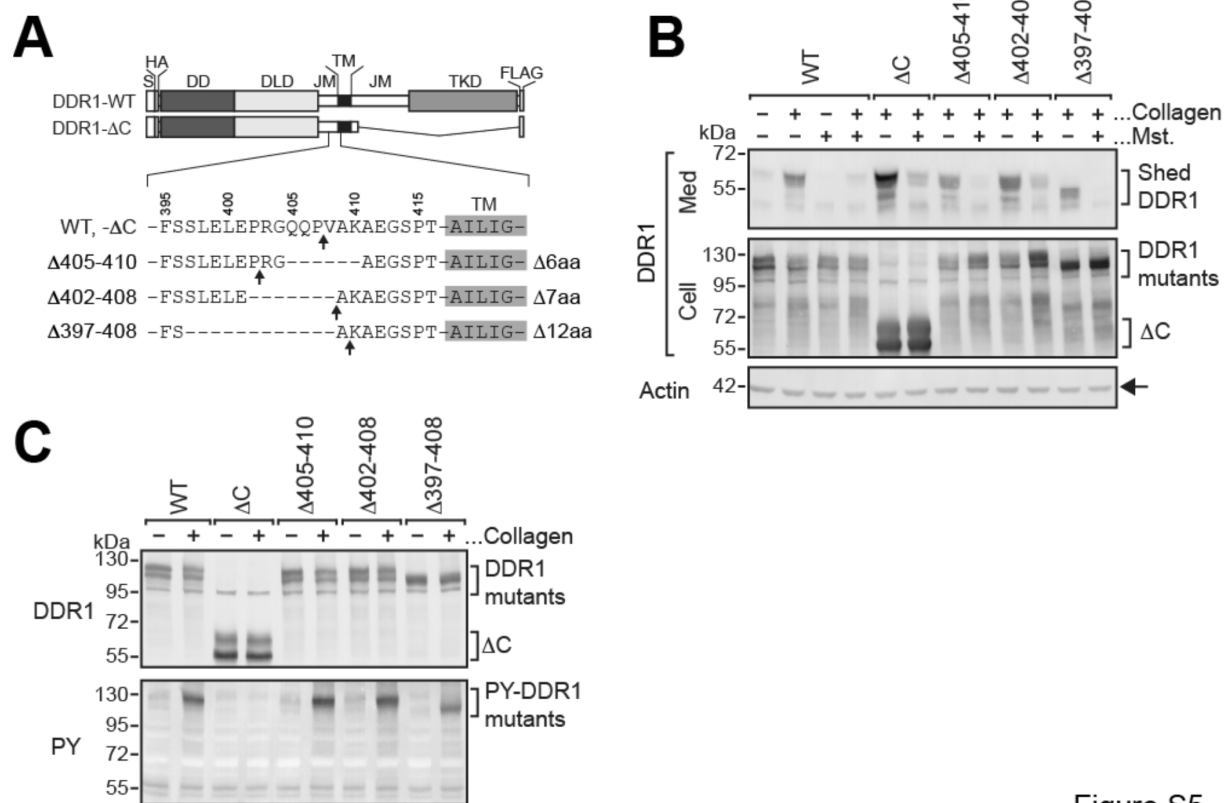


Figure S5

Substrate (type I or II membrane protein)	Protease	Cleavage site	Amino acids (aa) from TM
DDR1 (type I)			
DDR1-WT, Collagen-induced	ADAM10	-FSSLELEPRGQQPVAKAEGSPT <u>TAILIG</u> -	9 aa
DDR1ΔC, Collagen-induced	ADAM10	↑	
DDR1-WT, IM-induced	ADAM10	↑	
DDR1 mutants, Collagen-induced			
DAD	ADAM10	-FSSLELEPRGQQPDADAEGSPT <u>TAILIG</u> -	9 and/or 14 aa
6xD	ADAM10	↑↑-FSSLELEPRGDDDDDAEGSPT <u>TAILIG</u> -	17 and/or 19 aa
7xA	ND	↑-FSSLELEPRGAAAAAAEGSPT <u>TAILIG</u> -	9 aa
Δ405-410	ADAM10	↑-FSSLELEPRG-----AEGSPT <u>TAILIG</u> -	8 aa
Δ402-408	ND	↑-FSSLELE-----AKAEGSPT <u>TAILIG</u> -	8 aa
Δ397-408	ND	↑-FS-----AKAEGSPT <u>TAILIG</u> -	7 aa
EGF (type I)	ADAM10	↑-WWELRHAGH-	9 aa
E-cadherin (type I)	ADAM10	↑-RKAQPVEAG-	7 aa
N-cadherin (type I)	ADAM10	↑-TDVDRIVGA-	10 aa
FasL (type II)	ADAM10	↑↑-HTASSLEKQ-	24 and 27 aa
IL-6R (type I)	ADAM10, 17	↑-SLPVQDSSS-	8 aa
HB-EGF (type I)	ADAM10, 17	↑-GLSLPVENP-	11 aa
Notch (type I)	ADAM10, 17	↑-YKIEAVKSE-	15 aa
APP (type I)	ADAM10, 17	↑-VHHQKLVFF-	12 aa
TNF (type II)	ADAM17	↑-PLAQAVRSS-	20 aa
TGF-α (type I)	ADAM17	↑-ADLLAVVAA-	9 aa

ND, Not determined